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Stomatal Response of *Populus* Clones to Light Intensity and Vapor Pressure Deficit[1]

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ABSTRACT

Responses of stomata of clones of *Populus candicans* Ait. × *P. berolinensis* Dipp. and *Populus deltoides* Bartr. × *P. caudina* (Ten.) Bugala to two levels of light intensity and vapor pressure deficit were studied in controlled environments. Significant stomatal responses to light and vapor pressure deficit were observed. Interactive effects of low light intensity and high vapor pressure deficit elicited greater stomatal closure than was obtained under low light or high vapor pressure deficit alone, indicating adaptation for increased water use efficiency under conditions unfavorable for photosynthesis relative to transpiration. Adaxial stomata of both clones were more sensitive than abaxial stomata to changing vapor pressure deficit and light intensity. Stomatal response to vapor pressure deficit appeared to be independent of bulk leaf water status. Stomata of *P. candicans* × *P. berolinensis* were more sensitive than stomata of *P. deltoides* × *P. caudina* to a change in vapor pressure deficit and less sensitive to a change in light intensity. The sensitivity of stomata of *P. candicans* × *P. berolinensis* to vapor pressure deficit may be related to drought resistance in its parentage (*P. berolinensis*).

Response of stomatal aperture to environment modulates gas exchange between plants and their surroundings. The integrated response of stomata to many simultaneously acting influences still awaits adequate investigation. The evolution of this complexly controlled regulatory apparatus in such a wide array of plants emphasizes the importance of close control over water loss and CO_2 uptake to plant survival.

Stomatal response to light intensity has received wide attention (3). Stomatal closure at low light intensity or in darkness results in reduced water loss when the potential rate of photosynthesis is low or negligible. Recently, a direct effect of VPD[3] on stomatal aperture has been detected (13) and, in at least some cases, the response appears to be independent of bulk leaf water status (1, 14). Mechanisms have been advanced which propose an effect of ambient vapor pressure on water loss from guard cells resulting in altered guard cell turgor and, hence, change in stomatal aperture (8). Stomatal closure with increasing VPD might increase the WUE of plants because gas exchange would be more restricted when evaporative demand and potential transpiration rates were high (17).

Quantitative studies of interactive effects of changes in light intensity and VPD on stomatal aperture have been few (5, 12), and statistical treatment of interactive effects is lacking. Differential responses of stomata to VPD of certain species of plants have been invoked to explain differences in drought resistance (6, 19). The present experiment examined stomatal response to VPD and light intensity of two *Populus* clones of different parentage, growth rate, and morphology.

MATERIALS AND METHODS

Thirty-two plants of each of two *Populus* clones (*Populus candicans* Ait. × *P. berolinensis* Dipp., clone 5262; *Populus deltoides* Bartr. × *P. caudina* (Ten.) Bugala, clone 5267) were grown in the greenhouse. Mist-rooted tip cuttings were transplanted to 15-cm pots which contained an autoclaved soil mixture (3 parts sand: 2 parts loam). Plants were watered daily with tap water and weekly with full strength Hoagland solution.

Six weeks after transplanting eight plants of each clone were placed in each of four growth rooms in the University of Wisconsin Biotron. The plants were acclimated for 3 days with a 14-h photoperiod of 520 ± 30 μE m^{-2} s^{-1} PAR at the plant tops supplied by fluorescent and incandescent lights, 30/20 C ± 0.5 C day/night temperature and 1.32 ± 0.08 kPa VPD. The plants were watered twice daily with tap water throughout the experiment. After 3 days acclimation the following regimes of light intensity and VPD were imposed (one regime per chamber) at constant air temperature (30.0 ± 0.5 C) between 0700 and 1300 h: (*a*) 65 ± 8 μE m^{-2} s^{-1}, 0.78 ± 0.08 kPa; (*b*) 520 ± 30 μE m^{-2} s^{-1}, 0.78 ± 0.08 kPa; (*c*) 65 ± 8 μE m^{-2} s^{-1}, 3.16 ± 0.08 kPa; (*d*) 520 ± 30 μE m^{-2} s^{-1}, 3.16 ± 0.08 kPa. Acclimation conditions prevailed at other times in all chambers. Leaf temperature over all chambers during experimental treatment was slightly lower than air temperature, as estimated by the bead thermistor of a porometer sensor. Comparison of bead thermistor temperature with temperature measured by 0.08-mm copper-constantan thermocouples taped to abaxial leaf surfaces revealed an average difference of only 0.1 C. Leaf temperature averaged 28.9 C over all chambers with a maximum deviation of 0.5 C. Differences between leaf and air temperature were normally small (≤1 C); hence, VPD of the air and leaf to air VPD were nearly identical. Average leaf temperature differences between clones in a chamber were never greater than 0.1 C.

The porometer of Kanemasu *et al.* (10) and Lambda LI-65 Autoporometer electronics package were used to measure r_{AB} and r_{AD}. The porometer was calibrated in one of the growth rooms (light intensity: 65 μE m^{-2} s^{-1}; air temperature: 30 C; VPD: 0.78 kPa).

The experimental design was a 2 × 2 factorial split plot with

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[3] Abbreviations: VPD: vapor pressure deficit of the air; WUE: water use efficiency (g dry matter gained/g water transpired); r_l: diffusive resistance of a leaf surface; r_{AB}: diffusive resistance of the abaxial leaf surface; r_{AD}: diffusive resistance of the adaxial leaf surface; r_T: total leaf diffusive resistance calculated as the parallel sum of the abaxial and adaxial leaf diffusive resistances; ψx: negative xylem pressure potential; PAR: photosynthetically active radiation (400-700 nm).

Table I. Response of r_{AB}, r_{AD}, and r_T (s cm^{-1}), ratio of r_{AD} to r_{AB}, and ψx (MPa) of Populus clones 5262 and 5267 to two levels of both light intensity and VPD.

Mean values are shown ±1 SE.

Light intensity μE m^{-2} s^{-1}		VPD (kPa) 3.16		0.78	
		5262	5267	5262	5267
65	r_{AB}	8.04±0.86	13.02±0.92	3.15±0.22	7.20±0.67
	r_{AD}	51.08±4.09	29.42±2.88	8.92±0.89	17.13±2.41
	r_T	6.40±0.61	8.27±0.54	2.22±0.14	4.68±0.48
	r_{AD}/r_{AB}	7.78±0.62	2.38±0.17	2.88±0.17	2.32±0.17
	ψx	-0.41±0.01	-0.36±0.04	-0.45±0.05	-0.40±0.02
		5262	5267	5262	5267
520	r_{AB}	2.81±0.14	3.31±0.09	1.68±0.04	2.27±0.06
	r_{AD}	10.97±0.52	5.79±0.25	3.71±0.10	3.28±0.13
	r_T	2.19±0.09	2.05±0.05	1.14±0.02	1.32±0.04
	r_{AD}/r_{AB}	4.07±0.17	1.79±0.08	2.21±0.08	1.46±0.06
	ψx	-0.78±0.08	-0.72±0.09	-0.69±0.09	-0.71±0.06

light intensity and VPD treatments composing the main plots and clones the subplots (4). Because the variance of r_l increased with mean r_l a logarithmic transformation was performed on r_l data prior to analysis of variance. At 1000 h, after 3 h were allowed for stomatal equilibration under treatment conditions, r_{AB} and r_{AD} were measured on two fully expanded leaves of each plant. The procedure was repeated for each chamber. An evacuating gas mask was worn while a chamber was occupied to prevent exhaled CO_2 from accumulating inside. After r_l measurements were complete leaf water potential of one fully expanded leaf of each clone from each chamber was estimated by measuring its negative xylem pressure potential (ψx) with a pressure chamber (16). To maintain a constant sample population of leaves for r_l measurements, ψx was measured for leaves of similar age and exposure but other than those for which r_l data were obtained. To increase the precision of the experiment and compensate for effects of time of day on r_l, the entire procedure was repeated on the next three subsequent days and the sampling schedule was adjusted so that measurements were made on plants of each treatment at all four sampling times from 1000 to 1300 h.

Conversion of raw data to resistance was performed by a computer program. Total leaf resistance (r_T) was calculated as the parallel sum of r_{AB} and r_{AD} (15):

$$r_T = \frac{r_{AB} \times r_{AD}}{r_{AB} + r_{AD}}$$

RESULTS

Mean values of r_{AB}, r_{AD}, r_T, ratio of r_{AD} to r_{AB}, and ψx under four different light intensity-VPD combinations are shown in Table I. Significant differences in responses of r_l of *Populus* to light intensity and VPD and clonal differences in response to these factors are given in Table II. Leaf resistance of both clones responded significantly to changes in light intensity and VPD; interactive effects were also evident. Clonal differences in response of r_{AD} and r_T to VPD, light intensity, and their interaction were also significant. Abaxial, adaxial, and total r_l of clone 5267 showed a greater response to a change in light intensity compared with clone 5262. Adaxial and total r_l of clone 5262 were more responsive to increasing VPD than r_{AD} and r_T of clone 5267. A combination of low light intensity and high VPD resulted in greater changes in r_{AD} and r_T of clone 5262 when simple effects of low light and high VPD had been removed. Clonal differences in the response of abaxial stomata to VPD and interactive light intensity-VPD effects on r_{AB} were not significant.

Adaxial stomata of both clones were more sensitive to changes in light and VPD than were abaxial stomata, as indicated by changes in the ratio of adaxial to abaxial r_l. Clonal differences in the relative response of the stomata of the two leaf surfaces were also evident. Aperture of adaxial relative to abaxial stomata

Table II. Significance levels of F-tests of the analysis of variance of the effects of two levels of both light intensity and VPD on r_{AB}, r_{AD}, r_T and r_{AD}/r_{AB} of two Populus clones.

Effect	Significance level r_{AB}	r_{AD}	r_T	r_{AD}/r_{AB}
Light	**	**	**	*
VPD	**	**	**	**
Light x VPD	*	**	*	ns
Clone	**	**	**	**
Clone x Light	**	**	**	*
Clone X VPD	ns	**	**	**
Clone x Light x VPD	ns	**	**	**

** - signficant at the 1% level
* - signficant at the 5% level
ns - not significant at the 5% level

decreased with a reduction in light intensity more in clone 5267 than in clone 5262. Aperture of adaxial stomata relative to abaxial stomata decreased with an increase in VPD more in clone 5262. Under a combination of low light intensity and high VPD clone 5262 showed greater increase in relative adaxial:abaxial stomatal resistance after simple effects of low light and high VPD had been removed. The more sensitive subplot analysis detected interacting effects of light and VPD on r_{AD}/r_{AB} that were not brought out in the main plot analysis.

As VPD increased at high light intensity ψx decreased slightly, but larger decreases in ψx were obviated by increased stomatal closure in response to increasing VPD. At low light intensity, stomatal closure, induced by increased VPD, actually led to increased ψx despite a greater vapor pressure deficit. The influence of increased energy load and decreased r_l as light increased at both levels of VPD was reflected by lower ψx.

DISCUSSION

Both *Populus* clones exhibited significant changes in r_l when light intensity and VPD were varied, and both clones had an accentuated stomatal closing response to a combination of low light and high VPD over and above the sum of responses to either factor alone. These findings contrast with the observations of Sheriff (18) who found no stomatal response to humidity in *Populus alba,* but are consistent with positive responses of r_l to humidity observed in other species (7, 13).

The interacting effects of light intensity and VPD on r_l found in the present study have not been studied as thoroughly as responses of r_l to individual factors. Davies and Kozlowski (5) found that stomata of *Fraxinus americana* and *Acer saccharum* opened or closed more rapidly in response to an increase or a decrease in RH at 32,000 lux than at 6,500 lux. Kaufmann (12) observed that with similar humidity gradients, leaf conductance of *Picea engelmannii* was lower in leaves in the shade than in sun-

exposed leaves. The present study shows that a combination of low light and high VPD elicits a greater increase in r_l than can be accounted for by additive effects of the individual factors alone. This behavior emphasizes the capacity of *Populus* to reduce water loss sharply when conditions for CO_2 fixation in relation to water loss are exceptionally unfavorable. The response of r_l to high VPD and especially to low light intensity and high VPD suggest adaptation for increased WUE (8, 17).

Significant clonal differences in response of r_l to light intensity and VPD were observed. Total r_l of clone 5262 was more responsive to VPD. After simple factor effects had been removed, the interaction response of r_T of clone 5262 to low light and high VPD was greater than response of r_T of clone 5267. Most of the difference in clonal response to VPD was attributable to the high sensitivity to VPD of the adaxial stomata of clone 5262. The greater sensitivity of adaxial stomata of *Populus* to changes in VPD further supplements the observations that have been made concerning greater adaxial stomatal sensitivity to light intensity (11) and leaf water status (9).

The responsiveness of stomata of clone 5262 in reducing water loss under extreme evaporative stress may be related to drought resistance present in its parentage (2). Plantings of this clone may be preferred under situations where site and culture conditions require high drought resistance (*e.g.* unirrigated, sandy soils). Clone 5262 grows rather vigorously (much more so than clone 5267), and this may be at least partly related to its capacity to project a much larger leaf surface area and simultaneously protect it from extreme desiccation. The identification and manipulation of mechanisms that confer drought resistance in specific genetic material could be used in breeding programs to produce superior drought-adapted hybrids.

Clone 5262 might show better WUE than clones of other parentage which do not exhibit its degree of stomatal sensitivity to VPD (6, 17). However, we cannot conclude from the data that clone 5262 would show greater WUE under all circumstances, since WUE is determined by the balance between transpiration and photosynthesis, a balance that is greatly influenced by environmental conditions. In this experiment clones differed greatly in response of r_l to light intensity at low VPD. If photosynthesis of both clones was light-limited at 65 $\mu E\ m^{-2}\ s^{-1}$, clone 5267, which had a smaller r_l-VPD response and a larger r_l-light intensity response, might show greater WUE under low light conditions.

Increased WUE may not be the sole benefit of a direct response of r_l to VPD. As mentioned above, stomatal closure in response to increasing evaporative stress could be a homeostatic response, preventing lethal desiccation under the infrequent situations where the absorptive capacity of the plant cannot otherwise sufficiently offset unusually large transpirational demands.

The relatively moderate changes in ψx with a VPD increase at high light intensity and the increase in ψx with VPD increase at low light intensity, together with direct stomatal closure under each of these conditions, support the idea of a direct stomatal response to VPD independent of changes in bulk leaf water status (1, 14). Inasmuch as the present study did not include measurements of water relations of the epidermal cells and guard cells themselves, the possibility of stomatal closure at high VPD being attributable to localized water stress in the epidermis cannot be ruled out.

LITERATURE CITED

1. Bennett KJ, DA Rook 1978 Stomatal and mesophyll resistances in two clones of *Pinus radiata* D. Don known to differ in transpiration and survival rate. Aust J Plant Physiol 5: 231–238
2. Bugala W 1973 Systematics and variability. *In* S Bialobok, ed, The Poplars—*Populus* L. Nasze Drzewa Lesne Monografie Popularmonaukowe, Vol 12. US Dept Commerce Nat Tech Inf Serv, Springfield, Va, pp 7–108
3. Burrows FJ, FL Milthorpe 1976 Stomatal conductance in gas exchange control. *In* TT Kozlowski, ed, Water Deficits and Plant Growth, Vol 4. Academic Press, New York, pp 103–152
4. Cochran WG, CM Cox 1957 Experimental Designs, Ed 2. John Wiley & Sons, New York
5. Davies WJ, TT Kozlowski 1974 Stomatal responses of five woody angiosperms to light intensity and humidity. Can J Bot 52: 1527–1534
6. Hall AE, MR Kaufmann 1975 Regulation of water transport in the soil-plant-atmosphere continuum. *In* D Gates, R Schmerl, eds, Perspectives of Biophysical Ecology. Springer-Verlag, Berlin, pp 187–202
7. Hall AE, MR Kaufmann 1975 Stomatal response to environment with *Sesamum indicum* L. Plant Physiol 55: 455–459
8. Hall AE, ED Schulze, OL Lange 1976 Current perspectives of steady-state stomatal responses to environment. *In* O Lange, L Kappen, E Schulze, eds, Water and Plant Life: Problems and Modern Approaches. Springer-Verlag, Berlin, pp 169–188
9. Kanemasu ET, CB Tanner 1969 Stomatal diffusion resistance of snap beans. I. Influence of leaf water potential. Plant Physiol 44: 1547–1552
10. Kanemasu ET, GW Thurtell, CB Tanner 1969 Design, calibration, and field use of a stomatal diffusion porometer. Plant Physiol 44: 881–885
11. Kassam AH 1973 The influence of light and water deficit upon diffusive resistance of leaves of *Vicia faba* L. New Phytol 72: 557–570
12. Kaufmann MR 1976 Stomatal response of Engelmann spruce to humidity, light, and water stress. Plant Physiol 57: 898–901
13. Lange OL, R Losch, ED Schulze, L Kappen 1971 Responses of stomata to changes in humidity. Planta 100: 76–86
14. Macklon AE, PE Weatherly 1965 Controlled environment studies of the nature and origins of water deficits in plants. New Phytol 64: 414–427
15. Monteith JL 1973 Principles of Environmental Physics. Edward Arnold, London
16. Scholander PF, HT Hammel, ED Bradstreet, EA Hemmingsen 1965 Sap pressure in vascular plants. Science 148: 339–346
17. Schulze ED, OL Lange, M. Evenari, L Kappen, U Buschbom 1975 The role of air humidity and temperature in controlling stomatal resistance *Prunus armeniaca* L. under desert conditions. III. Effects on water use efficiency. Oecologia 19: 303–314.
18. Sheriff DW 1977 The effect of air humidity on water uptake by, and viscous flow resistance of, excised leaves of a number of species: physiological and anatomical observations. J Exp Bot 28: 1399–1407
19. Sheriff DW, PE Kaye 1977 Response of diffusive conductance to humidity in a drought avoiding and a drought resistant (in terms of stomatal response) legume. Ann Bot 41: 653–655